Channel regulation of glucose sensing in the pancreatic β-cell

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Hiriart M, Aguilar-Bryan L. Channel regulation of glucose sensing in the pancreatic β-cell. Am J Physiol Endocrinol Metab 295: E1298–E1306, 2008. First published October 21, 2008; doi:10.1152/ajpendo.90493.2008.—Mammalian β-cells are acutely and chronically regulated by sensing surrounding glucose levels that determine the rate at which insulin is secreted, to maintain euglycemia. Experimental research in vitro and in vivo has shown that, when these cells are exposed to adverse conditions like long periods of hypoglycemia or hyperglycemia, their capability to sense glucose is decreased. Understanding the normal physiology and identifying the main players along this route becomes paramount. In this review, we have taken on the task of looking at the role that ion channels play in the regulation of this process, delineating the different families, and describing the signaling that parallels the glucose sensing process that results in insulin release.

transient receptor potential; sodium current; calcium current; insulin secretion; insulin secretion coupling

Pancreatic β-cells secrete insulin in response to a “sensing” mechanism triggered by an increase in extracellular glucose from basal levels. Stimulation-secretion coupling in β-cells is different from other cell types because instead of being mediated by receptor binding, glucose needs to be transported into the cytoplasm and metabolized to stimulate exocytosis. Previous reviews (76, 51) have divided the glucose-sensing apparatus into proximal metabolic and distal ionic apparatus. The proximal component includes glucose transport through a specific transporter on the β-cell membrane (GLUT1 for humans, GLUT2 for rodents) followed by the glycolytic pathway, which results in an increase in the ATP/ADP ratio that triggers the distal component comprising a cascade of electrochemical events that culminate in an increase in intracellular calcium ([Ca²⁺]i) and stimulation of insulin secretion. This process can be augmented by several enteroinsular hormones [glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), and CCK] and neurotransmitters like acetylcholine, which amplify the secretory response through the activation of adenylyl cyclase or phospholipase C.

During fasting, basal glucose levels are ~5–6 mM, and the β-cell membrane is polarized at resting potential. Dean and Matthews (17, 18), almost four decades ago, were the first to show that when glucose rises voltage across the membrane oscillates, generating electrical activity leading to an increase in [Ca²⁺]i and subsequent insulin secretion. This activity consists of an initial slow membrane depolarization, followed by a fast depolarization and subsequent plateau level on which bursts of action potentials are superimposed to finally repolarize the plasma membrane, which returns to its initial polarized state at resting potential. This oscillating process regenerates as long as the glucose concentration is elevated and results from the activity of ion channels localized to the β-cell plasma membrane. Insulin is an anabolic hormone, essential for the maintenance of glucose homeostasis, because, among other effects, insulin increases glucose uptake in many cell types to promote nutrient storage. As a result, there is a decrease in glucose concentration that takes the system back to euglycemia and subsequent insulin cessation. This is a very tightly regulated process that when altered may result in hypoglycemia or hyperglycemia.

Several homeostatic mechanisms have been developed to make sure that euglycemia is maintained; therefore, stimulus-secretion coupling and agents or peptides modulating this mechanism play a very important role. Just to mention a few, it has been demonstrated that oral glucose promotes a more significant response than when glucose is applied directly into the circulation, and this is due to the glucose-dependent potentiation effect that incretins (GLP-1 and GIP) secreted by the gut have on insulin secretion. The nervous system also modulates insulin secretion through the effect of hormones/neurotransmitters like norepinephrine and epinephrine, which decrease exocytosis via α₂-receptors, or a muscarinic effect of acetylcholine, which stimulates the process. Finally, autocrine and paracrine regulation of glucose-induced insulin secretion has also been recognized. Autocrine effects are due on the one hand to insulin and nerve growth factor secreted by the β-cells acting on autoreceptors and, on the other hand, paracrine effects by glucagon and somatostatin secreted from α- and δ-cells within the islet, which respectively increase and decrease insulin secretion. Previous reviews on glucose sensing have focused mainly on the role that ATP-sensitive potassium (KATP) and voltage-dependent calcium (VDCC) channels play in the distal or ionic fraction of this process. In this minireview, we focus on the role of some other molecules that have not been previously carefully reviewed.
Concerns About the Different β-Cell Models

When speaking of pancreatic β-cells, it is difficult to generalize between the different existing models, because β-cells from different species behave in distinctive ways, reflecting the expression and diversity of proteins involved in glucose-stimulated insulin secretion (GSIS). In mammals, variation in secretory responses is determined by the type of meal and metabolic conditions of the animal. For example, certain ion channels from mouse β-cells are different from those expressed in rat. Human β-cells resemble rat and dog in their channel composition and secretory response more than mouse β-cells (11, 57). This is why investigators should be aware that direct comparisons of electrical activity and channel type between species may be difficult and create controversial data.

In addition, this comparison becomes more difficult because β-cell lines (HIT-T15, hamster; MIN6 and β-TC, mouse; INS-1 and RINm5F, rat) that secrete insulin are often used in research. These cells may not only diverge from normal β-cells in channel composition, but in other ways, including their glucose sensitivity, the magnitude of the insulin content and response to glucose, and the number of passages that can be subcultured before they become unresponsive to different stimul in addition to rapid cell division compared with normal β-cells that have a very slow turnover. Along the same lines, it has been shown that some of these cell lines, HIT-T15 and INS-1, when grown or selected in certain ways, may allow for a better and more representative model to work with. For example, when HIT-T15 cells are grown under low glucose concentrations, they can keep their glucose and insulin-secretory response at higher passages than when grown in 25 mM RPMI (71). In the case of the INS-1 cells, Hohmeier et al. (38), using clonal selection, were able to isolate a new line (832/13) with a robust response to glucose and other secretagogues in a similar fashion to the one seen using isolated islets.

Why do investigators use different models? Many of these studies are done using mouse β-cells because they are easy to isolate and currently the only suitable model for transgenic and knockout models. The same can be applied to many of the mouse diabetic models that investigators use to answer questions related to the pathophysiology of the disease.

Rats, which are larger in size, are a better model when taking a more physiological approach for clamp studies or in vivo perfused pancreas preparations. Finally, many investigators use β-cell lines because they are easier to culture and grow in large amounts for biochemical or molecular studies including ATPase activity or expression of mutant proteins, tasks that are very difficult to achieve in the rodent and human islet. Identifying the protein of interest in the system that we are working with and being informed of its limitations help us to achieve a better understanding of the process we are trying to characterize and a more homogeneous result compared with other studies published in the literature.

Glucose Stimulation-Insulin Secretion Coupling

The resting potential in β-cells is mainly due to the activity of ATP-sensitive potassium (K\(_{\text{ATP}}\)) channels and an electrogenic Na\(^{+}\)-K\(^{+}\)-ATPase pump that preserves the cationic gradient. When glucose is low or absent in the extracellular medium, the resting potential of isolated β-cells is around −70 to −80 mV. However, at basal glucose concentration it oscillates around −60 mV.

It is well accepted that when glucose concentration rises a first phase of coupling is observed, where glucose enters the cell and is metabolized, leading to a rise in the ratio of ATP to ADP (Fig. 1, in gray) and closure of K\(_{\text{ATP}}\) channels (20). These channels act as metabolic sensors, because they close in response to changes in the nucleotide concentration (ATP/ADP) that result from glucose metabolism. K\(_{\text{ATP}}\) closure initiates a slow depolarization of the membrane potential that, when it reaches around −40 mV, increases the open probability of Na\(^{+}\) and T-type Ca\(^{2+}\) voltage-dependent channels, increasing Na\(^{+}\) and Ca\(^{2+}\) entry into the cells and causing further depolarization. Around −20 mV, high-voltage Ca\(^{2+}\)-dependent channels open, and this leads to a larger increase in [Ca\(^{2+}\)], and subsequent insulin exocytosis (Fig. 1).

Insulin secretion in response to changes in extracellular glucose concentrations is biphasic; when extracellular glucose increases from basal to stimulating (10 mM glucose or more) a sharp and transient increase in insulin secretion is observed in the first few minutes and it is referred to as first phase. When high glucose concentrations are maintained, a second increase or phase is observed, which although lower in intensity than the first response, is sustained until euglycemia is achieved (31, 80, 99). The first phase of insulin secretion in response to glucose can be explained by the closure of the K\(_{\text{ATP}}\) channel and activation of the K\(_{\text{ATP}}\) channel-dependent or triggering signaling pathway depolarization that leads to the exocytosis of a small pool of granules (35). The mechanisms that underline the second phase seem to be more complex and a K\(_{\text{ATP}}\) channel-independent pathway of glucose signaling has been proposed (reviewed in Ref. 83), however, it is still controversial. Interestingly, β-cells lines like HIT-T15 or INS-1 only express the K\(_{\text{ATP}}\) channel-dependent pathway of glucose signaling (83).

K\(_{\text{ATP}}\) Channels: Structure and Function

The structure and function of these channels has been extensively reviewed (3, 21, 79, and references therein), so we will briefly describe the main characteristics, since a more detailed account is beyond the scope of this review.

K\(_{\text{ATP}}\) channels, initially described by Cook and Hales (1984) in pancreatic β-cells, have now been identified in multiple tissues, including neurons, smooth and skeletal muscle, and epithelial cells (15). These channels are unusual, because they form a hetero-octameric complex with proteins that belong to two very different families. The potassium-selective pore, K\(_{\text{IR6.1}}\) or -6.2, is a small inward rectifier with two transmembrane domains and a highly conserved pore region across K\(^{+}\) channels. The regulatory subunit or sulfonylurea receptor (SUR1 or -2) belongs to the ATP binding cassette (ABC) superfamily of transporters, with a predicted topology of 17 transmembrane domains (TMDs) (2, 87), divided into three main segments, TMD0, TMD1, and TMD2 and an important intracellular loop (Lo), which interacts with the inward rectifier for kinetics, gating, and sulfonylurea binding (7, 12).

The neuroendocrine K\(_{\text{ATP}}\) channel, composed of SUR1 or high-affinity SUR and the inward rectifier K\(_{\text{IR6.2}}\) (39), has been shown to be the same in the corresponding rat (RIN),
mouse (MIN), and hamster (HIT) cell lines and human β-cells. The key role that these channels play in the regulation of insulin release has been strengthened by the identification of mutations that give rise to two genetic diseases of infancy, hyperinsulinemic hypoglycemia (22) and neonatal diabetes (3).

To study functional channels in a reconstituted system, both subunits need to be expressed due to the presence of endoplasmic reticulum (ER) retention signals (97) that ensure only fully assembled, full-length channel traffic to the plasma membrane (80) and behave like native K_{ATP} channels. These two subunits assemble into a hetero-octameric complex where four SUR1 and four KIR6.2 associate in a 1:1 stoichiometry (14). The pore has K⁺ selectivity, determines the rectification, and is the gate that can be closed with ATP. SUR1 is responsible for nucleotide binding and hydrolysis at the nucleotide binding folds, and channel regulation by ATP and ADP requires cross talk between both subunits working in a balance between the inhibitory effect of ATP and the stimulatory effect of ADP. SUR1 has the binding sites for the pharmacological effects (6) of sulfonylureas (oral hypoglycemic agents) and K⁺ channel openers (diazoxide) that act as stimulators and inhibitors, respectively, of insulin release and therefore are used clinically to treat type 2 diabetes mellitus and hypoglycemic states.

By sensing glucose, K_{ATP} channels provide an extremely important link between cellular energetics and excitability that...
initiates a reduction in membrane potential and subsequent regulation of insulin secretion.

**Transient Receptor Potential Type Channels in β-Cells as Candidates for Depolarizing Currents**

The superfamily of transient receptor potential (TRP) proteins is composed of ionic channels that play important roles in multiple cellular processes, including the background current that contributes to membrane depolarization after K<sub>ATP</sub> channel closure and reloading of [Ca<sup>2+</sup>]<sub>i</sub>, stores and hormone secretion in vertebrates and invertebrates. TRPs are formed by six transmembrane segments and exhibit varying degrees of sequence homology and permeability/selectivity to cations, including calcium and magnesium.

Seven families of TRP channels have been described and classified using the following nomenclature: TRPC (canonical), TRPV (vanilloid), TRPM (polycystin), TRPML (mucolipin), TRPA (ankyrin), and TRPN, described only in flies and worms (90). Several of these channels have been identified as responsible for causing human diseases as well as contributing to the progression of several pathologies (48, 49, 61).

The activation of TRP channels can occur in several ways, in some systems by background currents already present in the cell, determined by channels that are constantly open, whereas in others they are activated by specific stimuli, like a rise in [Ca<sup>2+</sup>]<sub>i</sub>. Interestingly, several members of this superfamily of cation channels are known to mediate membrane depolarization in different cell types, which could be one of the functions they play in GSIS in pancreatic β-cells.

TRP channels are ubiquitously expressed, indicating that most cells have a different combination of TRP channel proteins that will determine the adequate function for that particular tissue. All types of pancreatic β-cells studied to date (rodent, dog, and human) as well as several cell lines (INS-1, HIT-T15, RINm5F, βTC3, MIN6) express a number of these channels where three types have been described: canonical (TRPC1, -4, -6), melastatin-like TRPM (TRPM2, -4, -5), and vanilloid-receptor-like TRPV (TRPV1), and it is thought that some of these TRP channels could provide a depolarizing background cationic current (44).

Taking advantage of the similarities in the calcium response to glucose (oscillations and [Ca<sup>2+</sup>]<sub>i</sub>) between the transgenically derived β-cells (βTC3-neo) and isolated rodent β-cells, some investigators have made use of unique experimental maneuvers that deplete intracellular calcium stores, e.g., the microsomal Ca<sup>2+</sup>-ATPase inhibitor thapsigargin (TG) or the activation of G protein-coupled receptors associated with the inositol triphosphate (IP<sub>3</sub>) cascade, to identify a current, referred to as Ca<sup>2+</sup> release-activated nonselcetive current (ICRAN) (72). In these cells, ICRAN can activate other nonselctive cationic current and some of the store-operated Ca<sup>2+</sup> channels that belong to the TRPC or TRPV family (reviewed in Ref. 69). INS-1 cells possess ryanodine-sensitive receptors (RYRs) in the ER, which could lead to the activation of TRP-like Ca<sup>2+</sup> channels that may depolarize the membrane to nearly −40 mV (30). At this voltage, the open probability of Na<sup>+</sup> and T-type Ca<sup>2+</sup> channels increases in β-cells, and currents through these channels can further depolarize the membrane.

Under resting conditions, primary rat β-cell membranes show a cationic current carried mainly by Na<sup>+</sup>, with a small contribution of Ca<sup>2+</sup>. Potentiation of this current by TG results in an increase in electrical activity and, thus, in calcium entry and insulin secretion at basal glucose concentrations (16). A possible candidate for this cationic influx is TRPM4, which is a channel, also permeable to Na<sup>+</sup> and Ca<sup>2+</sup>, that has been identified in murine, human, and β-cell line MIN-6, INS-1, HIT-T15, and RINm5F cells (13), that can lead to membrane depolarization and subsequent increases in [Ca<sup>2+</sup>]<sub>i</sub>, (reviewed in Ref. 45).

In native INS-1 cells, TRPM4 channels generate large depolarizing membrane currents in response to increased [Ca<sup>2+</sup>]<sub>i</sub>, which can be suppressed by expressing a dominant negative TRPM4 channel that results in decreased GSIS (13).

TRPM2 is a thermosensitive TRP channel that is present in human and rodent β-cells as well as RINm5F cells. These channels can be activated by cyclic ADP-ribose (cADPR), a product derived from nicotinamide adenine dinucleotide (β-NAD<sup>1+<sub>2</sub></sub>) in a reaction catalyzed by ADP-ribosyl cyclase (CD38) (86; reviewed in Ref. 25), which could be important in GSIS in β-cells. However, in pancreatic islets this channel cannot be activated at 25°C, but mild heating to 35°C or above increases the reaction and also [Ca<sup>2+</sup>]<sub>i</sub>, and insulin release. This process is activated as an alternative way to K<sub>ATP</sub> channel closure, and is mediated by protein PKA. Increases in cADPR evoke similar responses in β- and RIN-5F cells (86). The role of TRPM2 channels in apoptosis is linked to their activation by the free radicals produced by hydrogen peroxide, leading to irreversible cell depolarization and unregulated Ca<sup>2+</sup> entry into the cell. It has also been postulated as a mediator for alloxan destruction of β-cells (reviewed in Ref. 24).

Another monovalent-specific but nonselective cationic TRPM5 channel has been identified in the murine pancreatic β-cell line MIN6, in rat INS-1, and in human islets, but functional studies have been limited to cell lines. This channel represents a pathway for inward ion permeation that could also participate to the depolarizing current; however, more research is needed to clearly understand their role in rodent and human β-cells (67).

Although TRPC4 transcripts were readily identified in mouse pancreatic islets by use of RT-PCR, their importance has been questioned on the basis of the observation that glucose tolerance tests do not differ between wild-type and TRPC4-deficient mice (26); however, more studies are necessary to fully characterize the role that these channels are playing in GSIS.

TRPV1 has been shown to be present in both rat pancreatic neuronal fibers and islets in addition to RINm5F and INS-1 cells. The TRPV1 agonist capsaicin directly stimulated insulin secretion in RINm5F cells, and this effect was inhibited by capsazinpe, a TRPV1 antagonist, or by EDTA (4, 84). Studies looking at the expression of TRPV1 in the Zucker diabetic rat model of type 2 diabetes confirm their presence in the primary sensory fibers that innervate the islet and show that, when these channels are desensitized or eliminated with capsaicin, this contributes to the improvement in glucose tolerance due to an increase in insulin secretion (30). TRPV1 channels may also play a role in the nonobese diabetic (NOD) mouse altered glucose homeostasis through the innervation or the β-cells, but most probably both (70). These channels could also play an
important role in supporting the depolarization that takes place after closure of $K_{ATP}$ channels, contributing to the opening of VDCC.

**Calcium Oscillations and Different Calcium Channels in β-Cells**

Calcium movement in response to an increase in extracellular glucose, e.g., from 3 to 20 mM, involves a decrease in $[Ca^{2+}]_i$, followed by slow but large $Ca^{2+}$ oscillations and superimposed rapid calcium spikes. These $Ca^{2+}$ oscillations reflect the partial filling and emptying of $Ca^{2+}$ pools, particularly from the ER and an increase in $[Ca^{2+}]_i$, due to release from intracellular stores after glucose metabolism closes $K_{ATP}$ channels (27, 32, 33).

This last event would be important for the activation of an ICRAN carried by TRPM4 or TRPM5 that would further depolarize the plasma membrane.

The initial response is considered to be due to $Ca^{2+}$ entry into the ER, preparing β-cells to respond to different hormones and neurotransmitters, which will mobilize calcium from the ER via IP$_3$ channels or other calcium channels, like the ryanodine receptors (RyRs). The latter have been identified in several β-cells, but their specific role remains controversial. The mechanism by which a local rise in $[Ca^{2+}]_i$, becomes further amplified by calcium release by the ER is known as $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) (23) and could also be involved in the pulsatile nature of insulin secretion (reviewed in Ref. 40). Several studies propose that CICR in β-cells is mediated by RyRs that are present in all primary and tumoral β-cells studied so far (40), although other studies done in primary human β-cells, which express two types of RyRs, demonstrate that they can contribute only to glucose-indepedent control of insulin exocytosis (31, 47). The other route for $Ca^{2+}$ sequestration at the onset of glucose-induced changes in $[Ca^{2+}]_i$ is $Ca^{2+}$ uptake into mitochondria to fuel metabolism.

**Voltage-Sensitive Channels**

$Na^+$ channels can also contribute to membrane depolarization in GSIS. The initial work that was done on voltage-dependent $Na^+$ channels in pancreatic β-cells was controversial and concluded that $Na^+$ channels were not involved in either the plateau or spike generation because glucose-induced electrical activity was not affected by tetrodotoxin (TTX) (17, 56). In agreement, some of the initial patch-clamp studies done in mouse (73) and neonatal rat (75) indicated that few β-cells generate $Na^+$ currents. Subsequent studies reported that veratridine, which opens $Na^+$ channels in nerve cells, increased insulin secretion from perfused isolated rat islets (19) and depolarized rat β-cells (63), and both effects were blocked by TTX (28). The first demonstration of the importance of $Na^+$ currents present in rat β-cells was made by Hiriart and Matte-son (37), who also showed that this current is functionally important for stimulus-secretion coupling because TTX partially inhibits glucose-induced insulin secretion. TTX had no significant effect at or below 5 mM glucose, but at higher glucose concentrations TTX clearly inhibited the secretory response (37). Later, it was demonstrated that $Na^+$ channels are important for achieving a robust electrical activity and the highest insulin-secretory rate (91). Plant (66) in 1988 described for the first time the presence of $Na^+$ channels in mouse islets and concluded that it was unlikely that $Na^+$ currents played a role in glucose-induced electrical activity (66). The reason for this remark has to do with the observation that most $Na^+$ channels are inactivated or closed at resting potential, and the only way this inactivation can be removed is by hyperpolarizing the cell. $Na^+$ channels are present and participate in GSIS by depolarizing the membrane in canine and human β-cells (8, 11, 68).

$Na^+$ channels are glycoproteins formed by a principal α-subunit, which forms the pore that can be coupled to one or more β-subunits. Seven different genes coding for TTX-sensitive $Na^+$ channel α-subunits have been described (Nav1.1 to Nav1.7), which appear to play different roles depending on the cell type. β-Subunits regulate channel gating and cell surface expression of $Na^+$ channels. Nav1 channels are classified by their electrophysiological properties and their pharmacological sensitivity to a specific $Na^+$ channel blocker, TTX, at nanomolar concentrations.

Although all the β-cells studied to date express one or more types of TTX sensitive, voltage-dependent-$Na^+$ channels, at least two types of $Na^+$ channels have been characterized, Nav1.3 and Nav1.7 (65), in most β-cell types, and α-subunits Nav1.7 and Nav1.6 in human β-cells (11). However, it is not clear whether they are localized to different areas of the cell and the kind of role they may play.

Recently, the use of mathematical modeling supports the thought that $Na^+$ currents may be more relevant than previously thought, by analyzing the complex behavior of mouse β-cells upon stimulation with glucose, including repeated bursts and continuous spiking (55).

After $K_{ATP}$ closure and initial TRP-mediated depolarization, $Na^+$ channels open probability increases around −40 mV. $Na^+$ currents activate fast and transiently, with a maximum current around +10 mV. This current is completely inactivated 5 ms after it starts. Membrane repolarization or, even better, hyperpolarization removes $Na^+$ channel inactivation and leaves the channels ready to be reactivated with the next depolarization. When $Na^+$ enters the cell, the membrane is further depolarized and high-voltage-activated $Ca^{2+}$ channels open (37).

**VDCCs.** Native pancreatic β-cells and cell lines have different types of VDCC. These channels are classified according to their open probability in low-voltage-activated (LVA, type T channels) or high-voltage-activated (HVA, L, N, P/Q, and R). In both cases, they all form heteromeric complexes composed of α1, α2, β-, and γ-subunits. The α1 subunit forms the ion-conducting pore, and these channels have also been classified according to the α1 subunit being expressed. To date, at least 10 Ca$_{v1}$α1 genes and four distinct Ca$_{v1}$β genes (Ca$_{v1}$β1, Ca$_{v1}$β2, Ca$_{v1}$β3, and Ca$_{v1}$β4) have been identified/isolated from β-cells (reviewed in Ref. 96). β-Subunits in voltage-gated $Ca^{2+}$ channels are important auxiliary proteins for the functional expression of the channel complex, and they can also modulate α1 subunit activity. Rat and mouse primary β-cells express mRNA encoding more for β2 than for β3 subunits, and in the rat the β2 subunit is more abundant than β3 (41, 96).

Fasting blood glucose levels and basal insulin secretion in the β3 subunit-null mice are normal (9), but it has been reported that these mice present an augmented insulin response to glucose that results in better glucose homeostasis compared with wild-type mice. Further investigation is necessary to gain
a better understanding of the physiological role played by these subunits.

T-type Ca\(^{2+}\) channels or Ca\(V_{3.1}\) (\(\alpha_{1G}\)) are present in INS-1 cells of rat, dog, and human (11, 64, 98), but not in mouse (92). T-type channels are important in the regulation of insulin secretion and in triggering \(\beta\)-cell apoptosis in response to cytokines (94). In rat \(\beta\)-cells, T-type channels activate around \(-40\) mV and display a hump in the beginning of the whole cell \(I-V\) relationship, with a rapid inactivation and slow closing or deactivation (37, 74). In INS-1 and HIT-T15 cells, a T-type channel that is blocked by nickel has been detected. The activity of these channels may depolarize the membrane playing a pacemaker role, at postabsorptive glucose levels (10).

LVA T-type Ca\(^{2+}\) current was found in \(\beta\)-cells isolated from an NOD mouse; however, this type of current was not present in the nondoniabetic control mouse \(\beta\)-cells. This was associated with a higher [Ca\(^{2+}\)] in the NOD \(\beta\)-cells, and this factor may contribute to the pathogenesis of NOD mouse cells (93).

In comparison, when RINm5F and INS-1 cells were analyzed, it was shown that they also express T-type Ca\(^{2+}\) channels; although in these cells the activation occurs at more negative potentials, around \(-60\) mV. Human T-type channels also activate nearly at \(-60\) mV and peak around \(-30\) mV (11, 57, 96). It is possible that T-type channels, when expressed, contribute to the cell depolarization, opening around the same voltage that activates Na\(^{+}\) channels.

L-type Ca\(^{2+}\) channels are considered the most important Ca\(^{2+}\) entry pathway that controls insulin secretion in all the \(\beta\)-cells and cell lines studied. In general, these channels open in response to a strong depolarization, conduct Ba\(^{2+}\) better than Ca\(^{2+}\), and do not inactivate during long-lasting voltage pulses. Ca\(^{2+}\) channels are located near insulin granules in \(\beta\)-cells. One of the reasons for the biphasic time course of insulin secretion may reflect the sequential release of distinct pools of granules, which are associated with different types of L-type Ca\(^{2+}\) channels (95).

Rat and mouse express two different \(\alpha_1\) subunits in L-type Ca\(^{2+}\) channels, the \(\alpha_1D\) (Ca\(V_{1.3}\)) and \(\alpha_1C\) subunits (Ca\(V_{1.2}\)). There is wide agreement that, in rat, Ca\(V_{1.3}\) channels contribute the most to the insulin-secretory response. The mRNA level of Ca\(V_{1.3}\) is 2.5 times higher than that of Ca\(V_{1.2}\) (42). In addition, rat-derived INS-1 cells, wherein either \(\alpha_1D\) or \(\alpha_1C\) subunits were exclusively expressed, reveal that the \(\alpha_1D\) subunit is preferentially linked to GSIS (50, 62, 77). Interestingly, human \(\beta\)-cells also express Ca\(V_{1.3}\), and blockage of this L-type channel completely blocked insulin secretion (11, 57). In contrast, insulin secretion in mouse lacking the Ca\(V_{1.3}\) subunit was not affected (92). However, deletion of Ca\(V_{1.2}\) decreased [Ca\(^{2+}\)]\(_i\) to the same extent as the L-type channel blocker, indicating that it is the main type of Ca\(^{2+}\) channel present in these cells (7). In this model, nearly 80% of first-phase insulin secretion is inhibited, and these mice develop subsequent glucose intolerance.

It is possible that in mouse \(\beta\)-cells \(\alpha_1D\) is required for adequate regeneration during the postnatal period. Ca\(V_{1.3}\)-null mice were smaller at birth than their control littermates and exhibited hypoinsulinemia and glucose intolerance. However, glucose sensing and insulin secretion were normal, resulting from overexpression of the \(\alpha_1C\) subunit (Ca\(V_{1.2}\)). Even though the number of islets was the same in 1.3-null mice than in the wild-type, in adulthood they show less \(\beta\)-cell regeneration and display lower number and smaller islets than control littermates (58).

In the case of Ca\(V_{2}\) channels, the information available on their expression and function in \(\beta\)-cells is still small and controversial. When the \(\beta\)-cell line INS-1 was analyzed, it was shown that they express functional N-type Ca\(^{2+}\) channels, which play an important role in insulin secretion. This is not the case with RINm5F and HIT-T15 cells, where it is not clear whether they are present and functional in rat \(\beta\)-cells (reviewed in Refs. 82 and 96). Recent reports confirm that these channels are not present in human or mouse \(\beta\)-cells (11).

Ca\(V_{2.2}\) channels and the mRNA for the \(\alpha_1B\) subunit have been identified in adult rat \(\beta\)-cells, but only one-half of the channel proteins are localized to the plasma membrane, and the other half localizes to the cytoplasm (60). One possible explanation for this distribution could be that variability in extracellular cell conditions may determine the percentage of N-type channels present in the membrane.

PQ-type, Ca\(V_{2.1}\) (\(\alpha_1A\)), and Ca\(V_{2.3}\) (\(\alpha_1E\)) have been identified in all primary and tumoral cells studied to date (96). In INS-1 cells, where they have been further characterized, they do not seem to play an important role in GSIS (85). In human \(\beta\)-cells, blockade of PQ-type Ca\(^{2+}\) channels suppressed exocytosis, measured as a decrease in cell capacitance (11).

R-type channels have not been observed in human \(\beta\)-cells (11), but Ca\(V_{2.3}\) channels are present in rodent and INS-1 \(\beta\)-cells (29, 88, 89). This current is characterized by a faster inactivation than other HVA currents.

In the Ca\(V_{2.3}\)-null mice, the second phase of insulin secretion is reduced. These channels could be involved in recruiting insulin-containing granules from pools that are not immediately available to release their content (46, 95).

Despite the fact that neonatal and adult rats express the same type of Ca\(^{2+}\) channels, it is important to keep in mind that localization and number of channels is important for normal function. Rat neonatal \(\beta\)-cells are functionally immature because, compared with adults, they synthesize less insulin and their secretory response to glucose is lower; this effect has been associated with smaller Ca\(^{2+}\) currents, and although the secretory response improves after birth, by day 28 it is still less than in adults (1). Along the same lines it has been shown that mRNA levels for the \(\alpha_1\) subunits C and D are lower in neonates compared with adults, and this parallels the less abundant presence of channels in the plasma membrane (59). Interestingly, when mRNA levels for the accessory \(\beta_2\) subunit were investigated in the neonate, the results showed that it was 40% less abundant than in adults, suggesting that this subunit may promote \(\beta_2\) subunit translocation into the plasma membrane (59).

Returning to Resting Potential

Several types of voltage-gated K\(^+\) (Kv) and Ca\(^{2+}\)-sensitive voltage-dependent K\(^+\) (KCa\(^{2+}\)) channels have been described in all \(\beta\)-cells studied to date. These channels show similar dynamic properties to those recorded from neurons, and their activation is predominantly associated with repolarization. This area has been recently reviewed (54), so we will focus only on new observations. Although the initial description that Kv and KCa channel antagonist tetraethylammonium (TEA) prolongs mouse \(\beta\)-cell action potentials and increases insulin
secretion was made more than 30 years ago (5, 34), many
details about different K⁺ channels in β-cell physiology are
still lacking.

The functional unit of a Kv channel is a tetramer, assembled
of six transmembrane domain α-subunits, that forms the pore.
These channels have been detected in primary β-cells from rat,
mouse, pig, and human as well as from cell lines HIT-T15,
βTC3, INS-1, and RINm5F (54).

Kv channels are classified according to their pharmacolog-
ical behavior as follows. 1) KA-type current, identified in
mouse, rat, and MIN6 β-cells is blocked by 4-aminopyridine.
These channels activate and inactivate fast upon depolariza-
tion. KA currents are transient and present outward rectifica-
tion, but it is not clear whether they play a role in glucose-
induced electrical activity. 2) KCa, are Ca²⁺ sensitive and are
sub-classified according to their conductance in BK (for big)
and SK (for small). The BK calcium activated K⁺ channel is
present in all β-cells, and is thought to participate in repolar-
izing the plateau or the spikes (reviewed in Ref. 21). They
activate rapidly when the membrane is depolarized and are
very sensitive to an increase in [Ca²⁺]. 2) Kv2.1-type channels
are delayed rectifiers blocked by TEA, and when the mem-
brane is depolarized, these channels gradually activate and
inactivate slowly or not inactivate at all. It is thought that their
activity could be responsible for the repolarization of the fast
spikes in the electrical activity of β-cells. In rat and mouse,
Kv2.1 mediates the majority of the voltage-dependent outward
K⁺ current. A Kv2.1 channel, sensitive to external TEA, has
been identified in rat, mouse, pig, and human β-cells, as well
as in HIT-T15, βTC3, INS-1, and MIN6 cell lines (43, 52, 53).
Kv2.1-null mice, present increased insulin secretion that
results in lower fasting glucose levels or hypoglycemia com-
pared with wild-type mice (44). Electrophysiological ex-
periments using isolated β-cells show that Kv currents are ex-
tremely reduced compared with the wild-type, indicating that
Kv2.1 is the major component of Kv channels in mouse
β-cells. The duration of action potentials is increased, whereas
the firing frequency is decreased (44). Nearly 40 Kv
channel genes have been identified in humans, and β-cells
express a variety of them. Experiments using the whole cell
configuration established the presence of a delayed-rectifier
current in most of the human β-cells studied as well as a
transient, low-threshold A-current present in nearly 20% of
the cells (36).

Recently, two types of Kv currents that flow through delayed
rectifying (Kv2.1/2.2) and large-conductance Ca²⁺-activated
(BK) K⁺ channels have been described in human β-cells (11).In
that study, the importance of each channel type in GSIS was
addressed using specific toxins. Blockade of BK channels
increased action potential amplitude and enhanced insulin
secretion by 70%, whereas inhibition of Kv2.1/2.2 activity did
not show any stimulatory changes in electrical activity or
insulin secretion.

Closing Remarks

Secreting insulin as a result of changes in glucose levels is
a tightly regulated process that depends, among other things,
on the expression and orchestration of a very unique set of ion
channels that are present in the plasma membrane of the
pancreatic β-cell, which vary in the different β-cells that have
so far been characterized. These molecules can be regulated at
different levels, and current and future studies on the structure
and function should pave the way for a better understanding of
how failure of these pathways could cause diabetes mellitus,
keeping in mind that clear differences between models always
be taken into account.

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Review


